

WHAT IS CLAIMED IS:

1           1. A method of evolving a biocatalytic  
2 activity of a cell, comprising:

3           (a) recombining at least a first and second  
4 DNA segment from at least one gene conferring ability to  
5 catalyze a reaction of interest, the segments differing  
6 from each other in at least two nucleotides, to produce a  
7 library of recombinant genes;

8           (b) screening at least one recombinant gene  
9 from the library that confers enhanced ability to  
10 catalyze the reaction of interest by the cell relative to  
11 a wildtype form of the gene;

12           (c) recombining at least a segment from the at  
13 least one recombinant gene with a further DNA segment  
14 from the at least one gene, the same or different from  
15 the first and second segments, to produce a further  
16 library of recombinant genes;

17           (d) screening at least one further recombinant  
18 gene from the further library of recombinant genes that  
19 confers enhanced ability to catalyze the reaction of  
20 interest by the cell relative to a previous recombinant  
21 gene;

22           (e) repeating (c) and (d), as necessary, until  
23 the further recombinant gene confers a desired level of  
24 enhanced ability to catalyze the reaction of interest by  
25 the cell.

1           2. The method of claim 1, wherein the  
2 reaction of interest is the ability to utilize a  
3 substrate as a nutrient source.

1           3. The method of claim 1, wherein the  
2 reaction of interest is the ability to catabolize a  
3 compound.

1           4. The method of claim 1, wherein the  
2 reaction of interest is the ability to detoxify a  
3 compound.

1           5. The method of claim 1, wherein the  
2 reaction of interest is the ability to synthesize a  
3 compound of interest.  
4

1           6. The method of claim 4, wherein the  
2 compound is an antibiotic.

1           7. The method of claim 4, wherein the  
2 compound is an amino acid.

1           8. The method of claim 4, wherein the  
2 compound is a polymer.  
3

4           9. The method of claim 4, wherein the  
5 compound is a carotenoid.

1           10. The method of claim 4, wherein the  
2 compound is vitamin C.

1           11. The method of claim 4, wherein the  
2 compound is indigo.

1           12. The method of claim 1, wherein at least  
2 one recombining step is performed *in vitro*, and the  
3 resulting library of recombinants is introduced into the  
4 cell whose biocatalytic activity is to be enhanced  
5 generating a library of cells containing different  
6 recombinants.

1           13. The method of claim 12, wherein the *in*  
2 *vitro* recombining step comprises:  
3           cleaving the first and second segments into  
4 fragments;

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1 mixing and denaturing the fragments; and  
2 incubating the denatured fragments with a  
3 polymerase under conditions which result in annealing of  
4 the denatured fragments and formation of the library of  
5 recombinant genes.

1 14. The method of claim 1, wherein at least  
2 one recombining step is performed *in vivo*.

1 15. The method of claim 1, wherein the  
2 recombining step is performed in the cell whose  
3 biocatalytic activity is to be enhanced.

1 16. The method of claim 1, wherein at least  
2 one DNA segment comprises a cluster of genes collectively  
3 conferring ability to catalyze a reaction of interest.

1 17. A method of evolving a gene to confer  
2 ability to catalyze a reaction of interest, the method  
3 comprising:

4 (1) recombining at least first and second DNA  
5 segments from at least one gene conferring ability to  
6 catalyze a reaction of interest, the segments differing  
7 from each other in at least two nucleotides, to produce a  
8 library of recombinant genes;

9 (2) screening at least one recombinant gene  
10 from the library that confers enhanced ability to  
11 catalyze a reaction of interest relative to a wildtype  
12 form of the gene;

13 (3) recombining at least a segment from the at  
14 least one recombinant gene with a further DNA segment  
15 from the at least one gene, the same or different from  
16 the first and second segments, to produce a further  
17 library of recombinant genes;

18 (4) screening at least one further recombinant  
19 gene from the further library of recombinant genes that

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confers enhanced ability to catalyze a reaction of interest relative to a previous recombinant gene;  
(5) repeating (3) and (4), as necessary, until the further recombinant gene confers a desired level of enhanced ability to catalyze a reaction of interest.

18. A method of generating a new biocatalytic activity in a cell, comprising:

(1) recombining at least first and second DNA segments from at least one gene conferring ability to catalyze a first reaction related to a second reaction of interest, the segments differing from each other in at least two nucleotides, to produce a library of recombinant genes;

(2) screening at least one recombinant gene from the library that confers a new ability to catalyze the second reaction of interest;

(3) recombining at least a segment from at least one recombinant gene with a further DNA segment from the at least one gene, the same or different from the first and second segments, to produce a further library of recombinant genes;

(4) screening at least one further recombinant gene from the further library of recombinant genes that confers enhanced ability to catalyze the second reaction of interest in the cell relative to a previous recombinant gene;

(5) repeating (3) and (4), as necessary, until the further recombinant gene confers a desired level of enhanced ability to catalyze the second reaction of interest in the cell.

19. A modified form of a cell, wherein the modification comprises a metabolic pathway evolved by recursive sequence recombination.

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1 20. A method of optimizing expression of a  
2 gene product, the method comprising:

3 (1) recombining at least first and second DNA  
4 segments from at least one gene conferring ability to  
5 produce the gene product, the segments differing from  
6 each other in at least two nucleotides, to produce a  
7 library of recombinant genes;

8 (2) screening at least one recombinant gene  
9 from the library that confers optimized expression of the  
10 gene product relative to a wildtype form of the gene;

11 (3) recombining at least a segment from the at  
12 least one recombinant gene with a further DNA segment  
13 from the at least one gene, the same or different from  
14 the first and second segments, to produce a further  
15 library of recombinant genes;

16 (4) screening at least one further recombinant  
17 gene from the further library of recombinant genes that  
18 confers optimized ability to produce the gene product  
19 relative to a previous recombinant gene;

20 (5) repeating (3) and (4), as necessary, until  
21 the further recombinant gene confers a desired level of  
22 optimized ability to express the gene product.

1 21. The method of claim 20, wherein the at  
2 least one gene encodes the gene product.

1 22. The method of claim 20, wherein the at  
2 least one gene is a vector comprising a gene encoding the  
3 gene product.

1 23. The method of claim 20, wherein at least  
2 one recombining step is performed *in vivo*.

1 24. The method of claim 23, wherein the  
2 recombining step is performed in a host cell wherein the  
3 gene product is expressed.

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1           25. The method of claim 20, wherein the at  
2     least one gene is a host cell gene and wherein the host  
3     cell gene does not encode the gene product.

1           26. The method of claim 20, wherein  
2     optimization results in increased expression of the gene  
3     product.

1           27. A method of evolving a biosensor for a  
2     compound A of interest, the method comprising:

3           (1) recombining at least first and second DNA  
4     segments from at least one gene conferring ability to  
5     detect a related compound B, the segments differing from  
6     each other in at least two nucleotides, to produce a  
7     library of recombinant genes;

8           (2) screening at least one recombinant gene  
9     from the library that confers optimized ability to detect  
10    compound A relative to a wildtype form of the gene;

11          (3) recombining at least a segment from the at  
12    least one recombinant gene with a further DNA segment  
13    from the at least one gene, the same or different from  
14    the first and second segments, to produce a further  
15    library of recombinant genes;

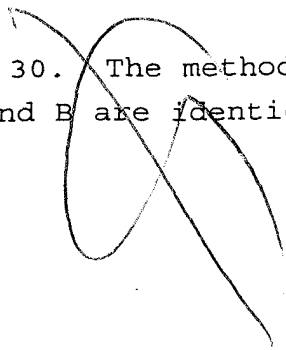
16          (4) screening at least one further recombinant  
17    gene from the further library of recombinant genes that  
18    confers optimized ability to detect compound A relative  
19    to a previous recombinant gene;

20          (5) repeating (3) and (4), as necessary, until  
21    the further recombinant gene confers a desired level of  
22    optimized ability to detect compound A.

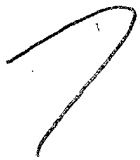
1           28. The method of claim 27, wherein  
2     optimization results in increased amplitude of response  
3     by the biosensor.

1           29. The method of claim 27, wherein compound A  
2     and compound B are different.

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- 1 30. The method of claim 27, wherein compound A  
2 and compound B are identical.
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